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LEAF ANATOMY OF FOURTEEN SPECIES OF *CALAMAGROSTIS* SECTION *DEYEUXIA*, SUBSECTION *STYLAGROSTIS* (POACEAE: POOIDEAE) FROM THE ANDES OF SOUTH AMERICA

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ABSTRACT

A scanning electron microscope and light microscope survey of the leaf epidermal and anatomical features in selected species of *Calamagrostis* from the Andes of South America was made for two purposes: first, to investigate any similarities or differences that may exist in the general and internal structure of the leaf of different species; and second, to investigate any structures of the leaf that may be associated with the environmental factors in these high altitude grasses. Abundant variation was found in such characters as the number of vascular bundles, number of ribs, patterns of distribution of sclerenchyma, shape and distribution of silica bodies, shape and distribution of epidermal papillae, leaf cross sectional outline, accumulation of silica in papillae and prickle hairs, stomata number and distribution, hair length, and hair distribution. The species studied exhibit anatomical and morphological diversification, which appears to be correlated with environmental factors.

KEY WORDS: grass leaf, leaf anatomy, Poaceae, *Calamagrostis*

INTRODUCTION

The genus *Calamagrostis* Adams. (Poaceae: Pooideae) with about 250 species is distributed worldwide (Bjoerkman 1969; Chase & Niles 1962; Clayton & Renvoize 1986). Approximately 70 poorly known species are found in the paramo and puna of South America. Studies based on microcharacters have shown that *Calamagrostis* is rather artificial (Hilu & Wright 1982), not appearing as a discrete cluster in any of their cluster analyses for the Gramineae.

It has been accepted that the anatomy of the leaf blade is an essential ingredient for a satisfactory analysis of grass taxonomy. The first person who pointed out that leaf anatomy might be useful in grass systematics was Duval-Jouve (1875), who found differences in bulliform cell distribution among species of different tribes and described two basic types of anatomy for grasses.

Many characters seen in the transverse sections of leaves appear to be quite constant and can be used with confidence in identifying grasses (Ellis 1976, 1979; Prat 1936). Other characters such as leaf size may vary with the habitat of the plant, but the basic form is genetically controlled (Humphries & Wheeler 1963).

Metcalfe (1960) described leaf anatomy for *Calamagrostis epigeios* (L.) Roth and *Deyeuxia quadriseta* Benth. He made a complete description, including leaf, stem, root, and geographical distribution for *C. epigeios.* For *D. quadriseta*, however, he described only the leaf epidermis and cross sectional anatomy. Tuerpe (1962) studied thirteen species of *Deyeuxia* in the province of Tucuman (Argentina). She considered two types of leaf anatomy:

1. having the bundles appressed to both lower and upper epidermis (e.g., *D. montevidensis* Nees, a species that grows in lower elevations [1000-2500 m]).

2. having their bundles isolated, few stomata, frequent epidermal hairs, and round silica bodies, (e.g., *D. eminens* Presl [*C. eminens* (Presl) Steud.], a species that grows at high altitudes [3000-5500 m]). According to Prat (1932, 1936), the anatomical characteristics of the *Calamagrostis* leaf resemble those of the Triticeae. Metcalfe (1960), based on his epidermal and anatomical studies of *Calamagrostis* and *Deyeuxia*, stated that the leaf is typically festucoid.

Metcalfe (1960) published the most comprehensive work describing the anatomy and epidermal characteristics for the entire grass family. He described the generic characters for *Deyeuxia* and *Calamagrostis* based on one species for each genus.

MATERIALS AND METHODS

Field collections and herbarium material were used for comparative studies of cross sectional leaf anatomy and epidermal characters. The leaf cross sections were cut from the midsection of the blade. Fully developed leaves from dried specimens were softened by soaking in Pohl's solution for seven days (Pohl 1965). After softening was complete, the leaf material was washed in tap water for fifteen minutes and then desilicified in a 10% aqueous hydrofluoric acid solution for nine days for paraffin sectioning. For further processing, the leaf pieces were rinsed in running water for three hours. Dehydration was accomplished in steps of 25%, 50%, 70%, 95% (two changes), and 100% (two changes) ethanol, with a minimum of one hour for each step.

Leaf samples to be embedded in paraffin were first stained in a solution of 1% safranin in 1:1 ethanol:xylene for one hour, and then passed through two changes of xylene before infiltration in melted wax (melting point 56.5 ° C) for one week. Sections were cut on a rotary microtome at 10 μm thickness, and stained in safranin and fast green using standard procedures (Berlyn & Miksche 1976; Sass 1958). Living leaf blades were cut in water and stained following the procedure for making permanent free hand cross sections (cover slip was sealed using two coats of clear nail polish).

For scanning electron microscope (SEM) observations, the leaf epidermis and floret samples were selected under a binocular microscope. Leaf samples were selected by cutting square or rectangular sections from the midposition of mature foliage leaves. Complete florets and leaf samples were mounted on brass discs with silver paste or silver tape, coated with Au/Pd in a Polaron E5100 sputter coater, and viewed at 15 and 39 Kv in a Jeol JSM-35 Scanning Electron Microscope. To observe features such as silica bodies, cork cells, stomata, bulliform cells, and papillae more clearly, leaf sections were sonicated in xylene for 12-15 minutes to remove the epicuticular wax, then allowed to air dry before mounting. Photographs were taken using Polaroid type 665 positive/negative film. Elemental X-ray analysis for silicon was performed using a Kevex-ray subsystem 5000A X-ray energy spectrometer attached to the scanning electron microscope. Special observation of the adaxial epidermis under the scanning microscope was made for those specimens showing considerable contrast differences (deep furrows and elongated ribs) on the adaxial epidermis, by using "gamma control unit" to optimize the image contrast by decreasing the contrast in high contrasted areas (ribs) and increasing the contrast in low areas (furrows) (Horner & Elsner 1981).

RESULTS AND DISCUSSION

Anatomical description: subsection *Stylagrostis*. Leaf thickness was measured in various units or ribs with an average thickness of 0.5-1.0 mm. Transverse sections in normally permanently of temporarily infolded leaves exhibit reduced V shaped, U shaped, or round outline (Figs. 1e, 1f, 1g, 1h, 1i, 1j, 1k, 1l, 1m, and 1n). *Adaxial furrows*: from slight, shallow to deep, varying in shape from wide to narrow, and distributed between the vascular bundles. *Adaxial ribs or units*: situated over the vascular bundles with flat tops as in *Calamagrostis pisinna* Swallen (Fig. 7); rounded tops alternating with triangular tops as in *C. ampliflora* Tovar (Fig. 5); triangular tops as in *C. ovata* (Presl) Steud. (Fig. 6). *Abaxial furrows and ribs*: not present. *Median vascular bundle*: present but sometimes not distinguishable from other primary vascular bundles. Usually the leaf infolding occurs in the medial furrow or rib with no structurally distinct midrib projecting abaxially (Fig. 1). Frequently, the central primary vascular bundle is surrounded by a large group of

parenchyma (Fig. 7) or thick walled cells (Fig. 5) and/or sclerenchyma (Figs. 2 and 7). *Vascular bundle arrangement*: first order vascular bundles present, varying in number. The xylem of first order vascular bundles is characterized by large metaxylem vessels on either side of the protoxylem (Figs. 2 and 3). The vascular bundles may be circular (Fig. 7), ovate (Fig. 5), or apple shaped (Fig. 3). The phloem is sometimes sclerosed, connected or not to lignified fibers. *Second order vascular bundles*: usually present, round or ovate, xylem and phloem, well differentiated, sometimes the same size as first order vascular bundles but lacking large metaxylem vessels. *Third order vascular bundles*: sometimes present, mostly bearing phloem and lacking bundle sheaths. *Vascular bundle sheaths*: a double vascular sheath surrounding each vascular bundle, not distinguishable in third order vascular bundles. The outer or parenchyma sheath cells are well differentiated from the chlorenchyma cells, sometimes interrupted by sclerenchyma (Fig. 2) or thick walled cells (Fig. 5). The inner, or mestome, sheath is complete or interrupted by sclerenchyma girders, cells relatively large with inner tangential and radial cell wall thickening (Figs. 4 and 7). The cells of the inner sheath adjacent to the xylem are larger; the cells of the inner sheath adjacent to the phloem fibers are smaller and sometimes not distinguishable from the latter. *Adaxial and abaxial sclerenchyma*: adaxial sclerenchyma associated with the vascular bundles occurs as strands or girders. The strands are not in contact with the vascular bundle sheaths. They are separated by mesophyll (chlorenchyma or colorless parenchyma thick walled cells) (Fig. 3). Girders can be in contact with or interrupting the bundle sheath (Fig. 7). Both strands and girders can be present or absent. In permanently infolded leaves, the sclerenchyma may be exhibited as follows: abaxial triangular strands opposite vascular bundles, e.g., *C. eminens* (Fig. 1i); continuous abaxial subepidermal layers, not connected to the vascular bundles by girders, e.g., *C. chrysantha* (Presl) Steud., *C. amoena* (Pilger) Pilger, *C. aurea* (Munro) Hack. (Figs. 1f, 1e, and 1n); continuous, abaxial, subepidermal layers connected to bundles by girders, e.g., *C. ampliflora* (Fig. 1a); continuous, abaxial subepidermal layers connected to bundles by girders, and girders connected to bundles from the adaxial surface, e.g., *C. mollis* Pilger (Fig. 1h). *Sclerenchyma between bundles*: sclerenchyma present or absent between vascular bundles. When present, it occurs as strands of hypodermal layers with girders extending to vascular bundles or not (Figs. 1d, 1e, 1i, 1j, 1l, and 1n). *Sclerenchyma in leaf margin*: present or absent; when present, cape or hood shaped, presenting ranges of size and shape (Figs. 1f and 1h). *Mesophyll*: the chlorenchyma is composed of isodiametric or irregularly shaped cells, sometimes with air spaces. In some species, the chlorenchyma constitutes a relatively small part of the whole unit, arranged in layers following the shape of the ribs and furrows, as in *C. ampliflora* (Figs. 1a and 5) and *C. chaseae* Luces (Figs. 1l and 2), with the rest of the mesophyll filled with thick walled, colorless parenchyma cells or sclerenchyma. There is no differentiation

between palisade and spongy parenchyma (Fig. 7). Thick walled parenchyma or elongate cells can be present or lacking in the leaf mesophyll. When they are present, the parenchyma cells can be localized over the vascular bundles, forming an arch or continuous girder from the vascular bundle sheath to the adaxial epidermis (Fig. 2). The bulliform cells are restricted to the furrows on the adaxial surface, with a very thin wall. The number of bulliform cells is 5-8, conspicuously large or well defined cells gradually larger than the rest of the epidermal cells (Fig. 4).

The microscopic anatomical examination of species of subsection *Stylagrostis* was made to investigate the similarities and differences that may exist in the general and detailed internal structure of different species in leaf cross section in order to relate leaf structure to ecological characteristics. As was shown by Tuerpe (1962), altitudinal differences determined two types of leaf anatomy, based mainly on vascular bundle position with respect to the epidermis. Anatomy of the leaf blade in some species of subsection *Stylagrostis* agreed with what was found by Tuerpe, but the papillae which are a very important adaptation for some species, such as *Calamagrostis eminens, C. ovata, C. chrysantha*, etc., were not mentioned. Round silica bodies have been described for the species within subsection *Stylagrostis*. Some species have large silica cells with sinuous edges, located over the top of the ridges. Also, *C. mollis* was the only species found to exhibit long hairs. Leaf anatomy of species of subsection *Stylagrostis* is variable, but typically festucoid as was described by Gould & Shaw (1983), Metcalfe (1960), and Prat (1932).

Scanning electron microscope surveys of leaf anatomy and epidermis have brought to light anatomical details that were not previously discernible by light microscopy. Such surveys have provided valuable information for plant taxonomists. Agrostologists have shown the importance of such studies in classifying living and fossil plants (Albers 1980; Flores, Espinoza, & Kosuka 1977; Hilu 1984; Hilu & Wright 1982; Maeda & Miyake 1973; Palmer & Tucker 1981, 1986; Palmer, Gesbert-Jones, & Hutchinson 1985; Terrell & Wergin 1979, 1981; Thomasson 1978a, 1978b, 1980a, 1980b, 1981, 1984, 1986). Silica accumulates in silica bodies contained in silica cells (Gould & Shaw 1983; Parry & Smithson 1964). Scanning electron microscope studies also show that silica accumulates in other epidermal structures such as prickles (Sakai & Sanford 1984; Terrell & Wergin 1981), bulliform cells (Dayanardan, Kaufman, & Franklin 1983; Parry 1958), and the stomatal apparatus (Sakai & Sanford 1984).

Stomata are usually located at the bases and sides of the furrows on the adaxial epidermis, rarely at the top of the ribs (*Calamagrostis cleefii* Escalona), (Fig. 10) associated or unassociated with papillae (Figs. 23, 24, 25, and 38). The stomata are generally arranged in longitudinal rows separated by files of costal or intercostal cells (Figs. 8, 12, 13, and 41). Usually, there is one interstomatal cell between successive stomata (Figs. 8, 9, 12, 13, and 38). The

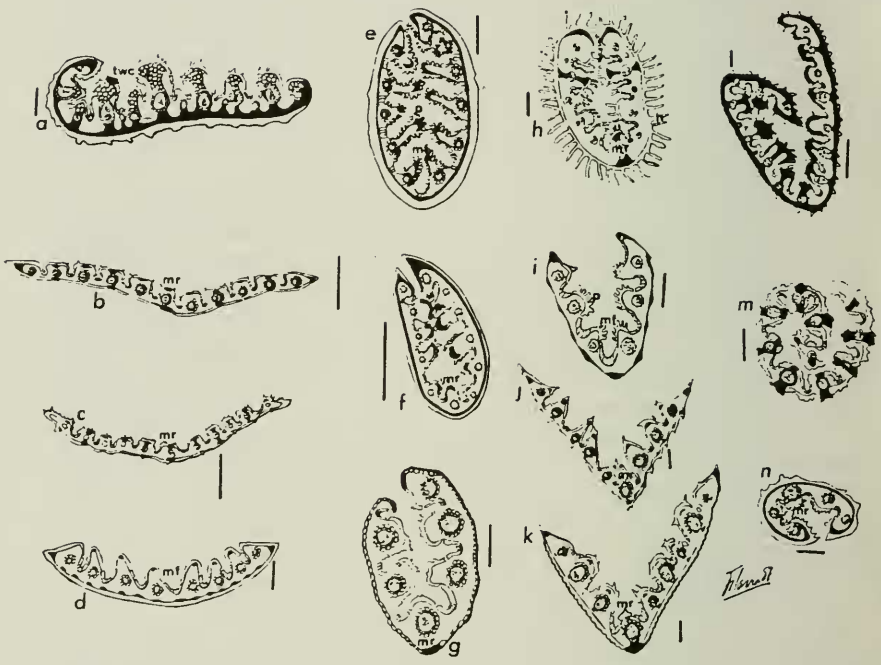
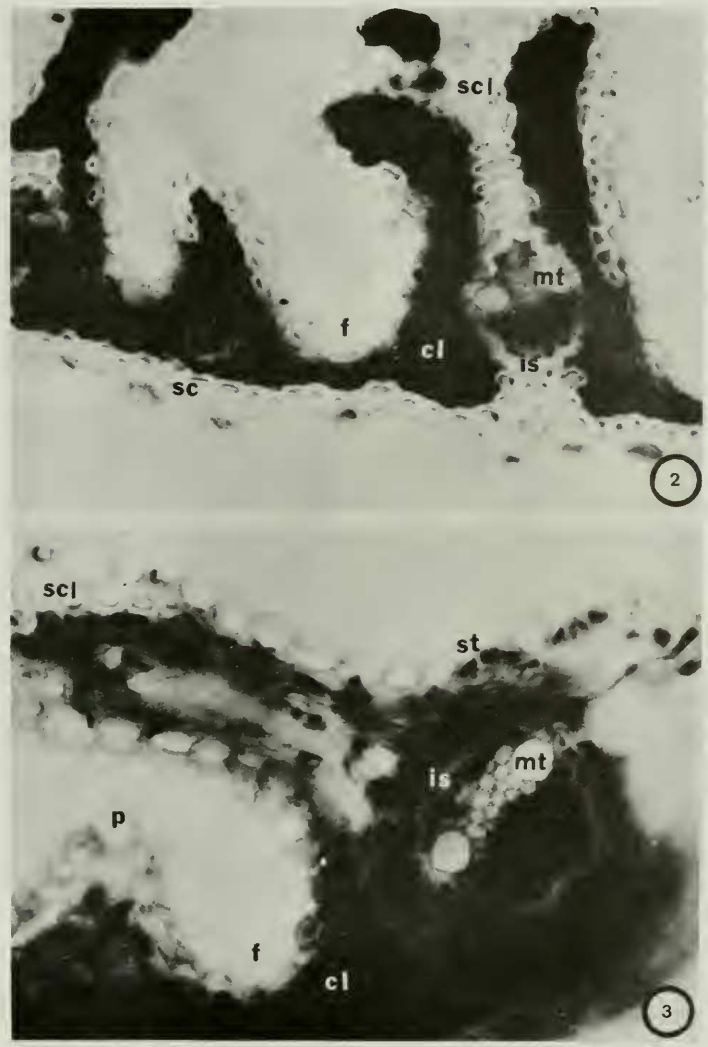
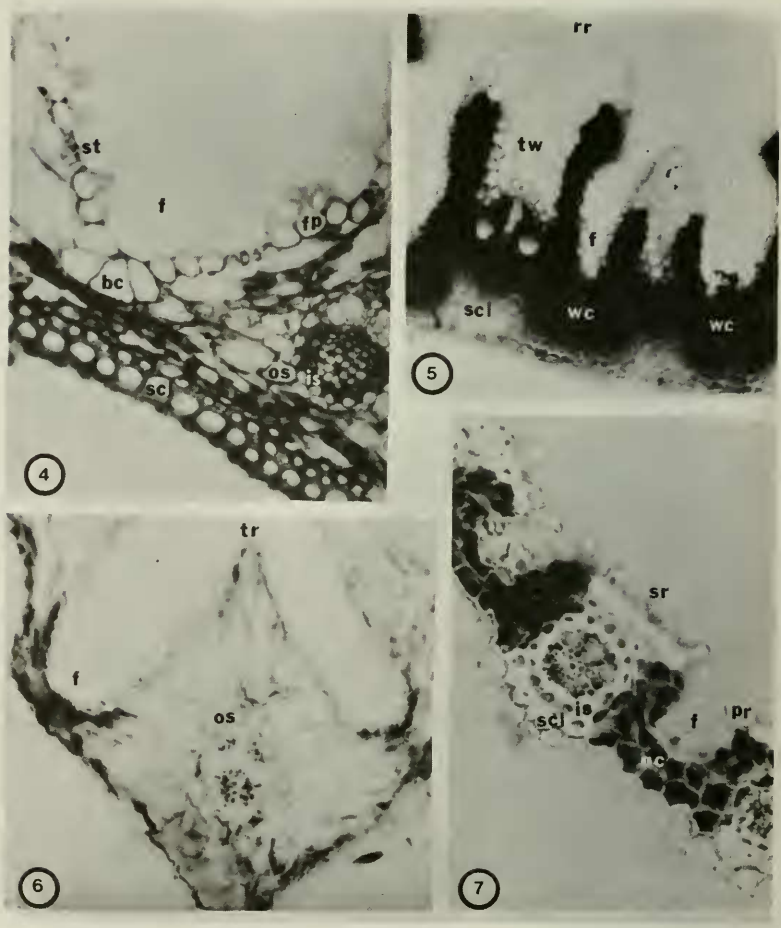


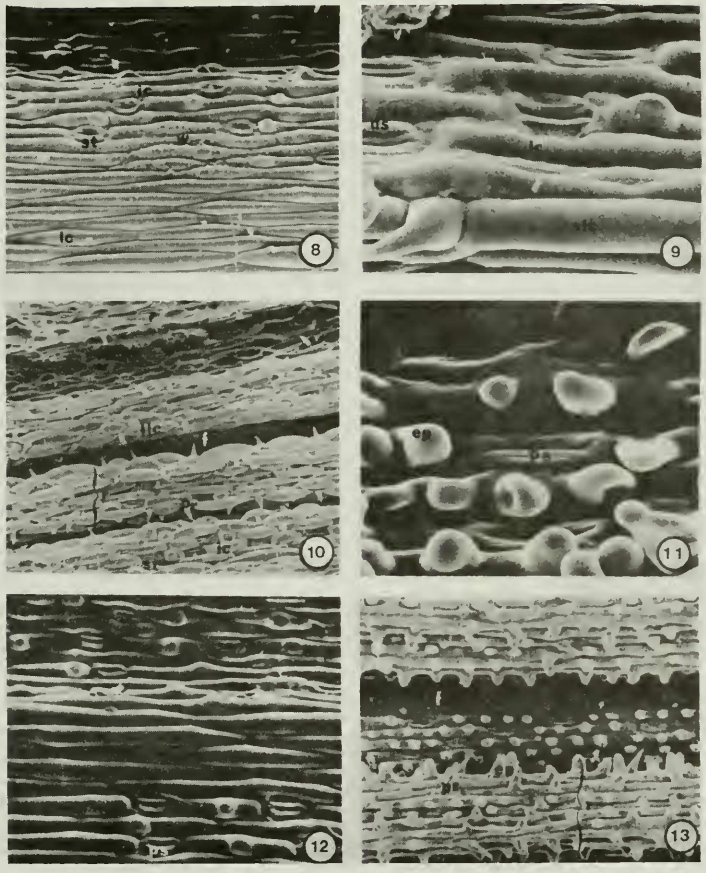
Figure 1. Leaf outline and anatomical structure. Dark areas represent sclerenchyma, white areas represent chlorenchyma, md = midrib, mf = midfurrow, p = papillae, h = long hairs, txc = thick walled parenchyma cells. a) *Calamagrostis ampliflora*, from *Hitchcock 22327*, bar = 2.5 mm; b) *Calamagrostis guamanensis* Escalona, from *Escalona et al. E390*, bar = 5.6 mm; c) *Calamagrostis ramonae* Escalona, from *Steyermark 55903*, bar = 4.3 mm; d) *Calamagrostis ligulata* (H.B.K.) Hitchc., from *Ollgaard 10772*, bar = 1.3 mm; e) *Calamagrostis chrysantha*, from *Escalona et al. B566*, bar = 1.0 mm; f) *Calamagrostis aurea*, from *Acosta Solia 7223*, bar = 4.0 mm; g) *Calamagrostis cleefii*, from *Cleef 7768*, bar = 1.0 mm; h) *Calamagrostis mollis*, from *Asplund 8400*, bar = 5.3 mm; i) *Calamagrostis eminens*, from *Escalona et al. B669*, bar = 1.0 mm; j) *Calamagrostis ovata*, from *Turner et al. 1312*, bar = 1.0 mm; k) *Calamagrostis curta* (Wedd.) Hitchc., from *Solomon et al. 11654*, bar = 1.0 mm; l) *Calamagrostis chaseae*, from *Luces 292*, bar = 2.0 mm; m) *Calamagrostis pisinna*, from *Burandt et al. V0401*, bar = 1.0 mm; n) *Calamagrostis amoena*, from *Lara et al. 21f*, bar = 0.7 mm;



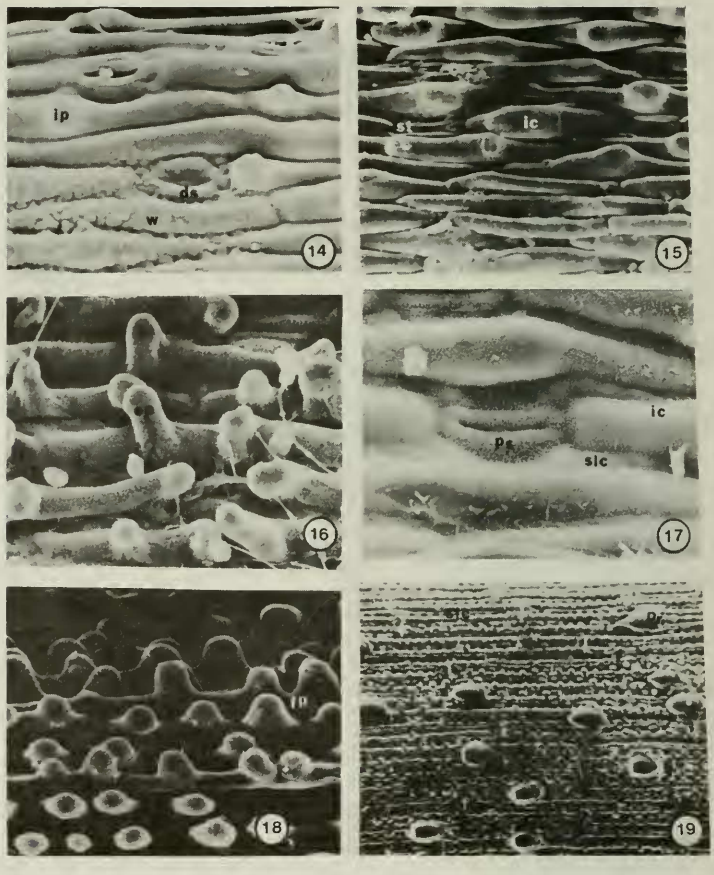
Figures 2 and 3. Leaf blade cross sections from species of subsection *Stylagrostis*. cl = chlorenchyma, f = furrow, is = inner sheath, mt = metaxylem, p = papilla, scl = sclerenchyma. 2) *Calamagrostis chaseae*, from *Briceño 229* (X 420). 3) *Calamagrostis chrysantha*, from *Escalona et al. B566* (X 560).



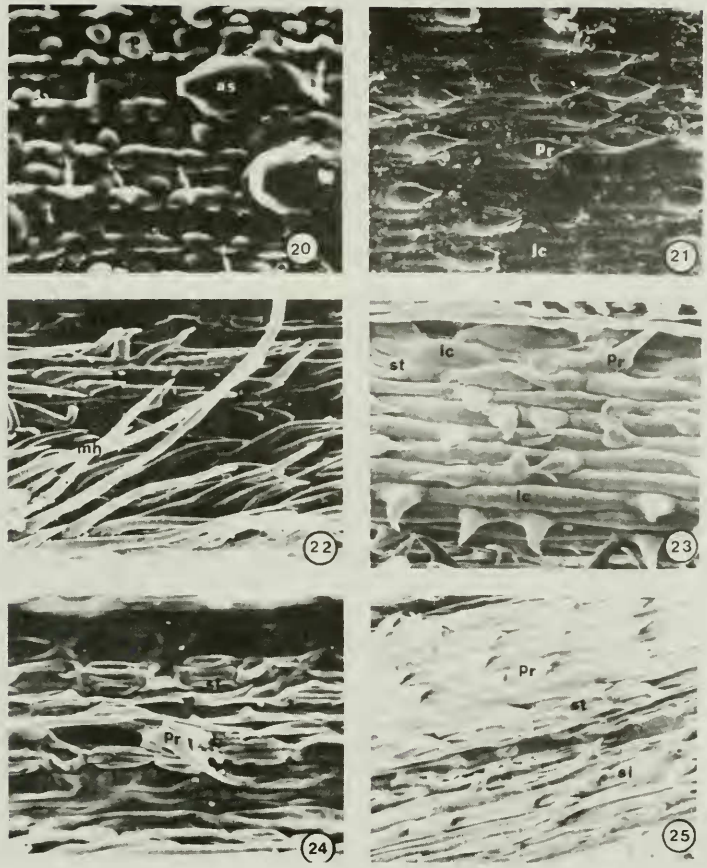
Figures 4-7. Leaf blade cross sections from species of subsection *Stylagrostis*. bc = bulliform cells, cl = chlorenchyma, f = furrow, fp = forked papilla, is = inner sheath, mt = metaxylem, os = outer sheath, p = papilla, pr = prickle, rr = round constricted rib, scl = sclerenchyma, sr = square rib, st = stomata, tr = triangular rib, tw = thick walled parenchyma cells, uc = u shaped chlorenchyma, wc = w shaped chlorenchyma. 4) *Calamagrostis eminens*, from *Escalona & D. Smith P420* (X 700). 5) *Calamagrostis ampliflora*, from *Hitchcock 22327* (X 480). 6) *Calamagrostis ovata*, from *Escalona et al. B547* (X 420). 7) *Calamagrostis pisinna*, from *Escalona & Escalona 229* (X 360).



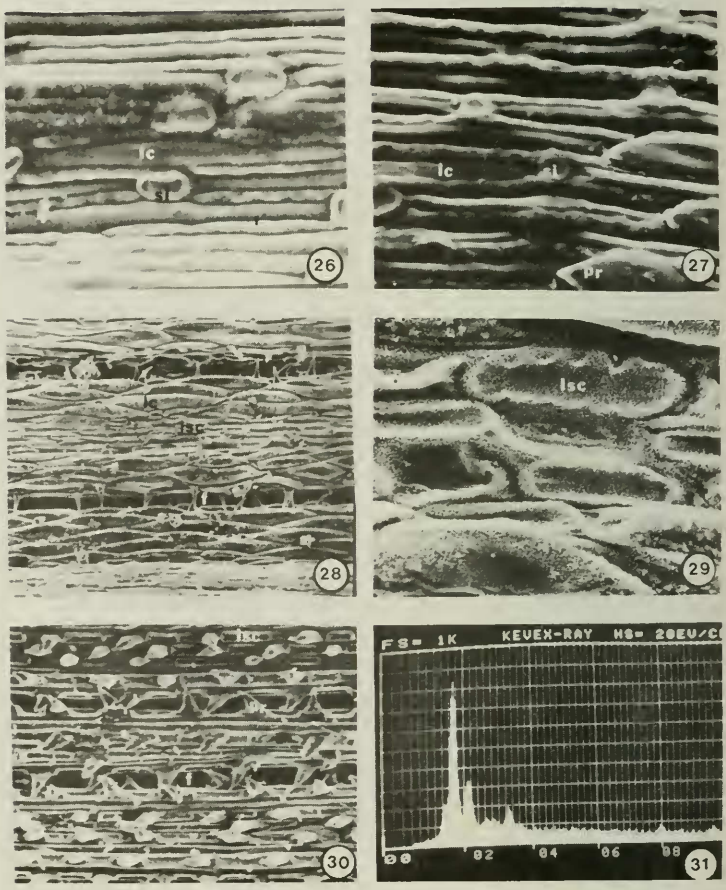
Figures 8-13. Scanning electron micrographs of adaxial epidermis. * = material treated with xylene, # = scanning gamma technique, ds = low dome shaped subsidiary stomata cell, ep = elongated papilla, f = furrow, ic = interstomatal cell, ilc = inflated long cell, pr = prickle, ps = parallel sided subsidiary stomata cell, r = rib, slc = straight edged long cell, st = stomata, w = wax. 8) *Calamagrostis aurea*, from *Asplund E7943* (X 260), notice waxy surface #. 9) *Calamagrostis ovata*, from *D. Smith & Escalona 19177* (X 720), notice waxy surface. 10) *Calamagrostis cleefii*, from *Cleef 7768* (X 160). 11) *Calamagrostis chrysantha*, from *Tovar 2530* (X 1100). 12) *Calamagrostis aurea*, from *Jameson 95* (X 300) #*. 13) *Calamagrostis eminens*, from *Lillo 5045* (X 220).



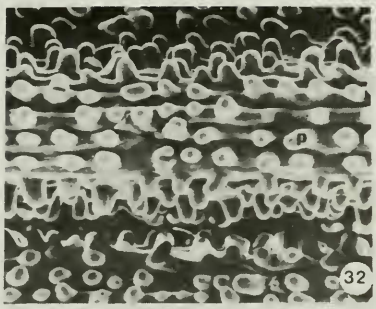
Figures 14-19. Scanning electron micrographs of adaxial or abaxial epidermis. # = scanning gamma technique, dst = low dome shaped subsidiary stomata cell, ep = elongated papilla (4-6 per cell), f = furrow, fp = forked papilla, ic = interstomatal cell, ilc = inflated long cell, ip = inflated papilla, pr = prickle, ps = parallel sided subsidiary stomata cell, slc = sinuous edged long cell. 14) *Calamagrostis aurea*, from *Asplund E7943* (X 660) #. 15) *Calamagrostis ovata*, from *Escalona et al. B554* (X 440) #. 16) *Calamagrostis chrysantha*, from *Escalona et al. B549* (X 940) #. 17) *Calamagrostis ovata*, from *Escalona et al. B554* (X 940). 18) *Calamagrostis chrysantha*, from *Escalona et al. B566* (X 940). 19) *Calamagrostis ovata*, from *Escalona et al. B566* (X 240), abaxial epidermis.

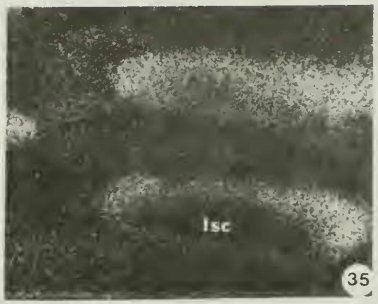


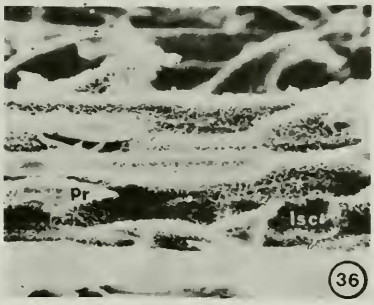
Figures 20-25. Scanning electron micrographs of adaxial or abaxial epidermis. * = material treated with xylene, # = scanning gamma technique, as = asperite, lc = long cell, mh = macrohair, p = papilla, pr = prickle, si = silica cell, st = stomata. 20) *Calamagrostis eminens*, from *Solomon 12140* (X 940), abaxial epidermis. 21) *Calamagrostis ampliflora*, from *Hitchcock 22327* (X 200), abaxial epidermis. 22) *Calamagrostis mollis*, from *Escalona & D. Smith 465* (X 310). 23) *Calamagrostis ampliflora*, from *Hitchcock 22327* (X 440). 24) *Calamagrostis curta*, from *Solomon et al. 11654* (X 480) *. 25) *Calamagrostis amoena*, from *Escalona & Solomon B683* (X 320) #.

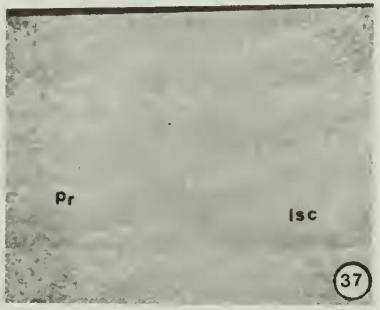


Figures 26-31. Scanning electron micrographs of adaxial or abaxial epidermis, and silica spectrum. f = furrow, lc = long cell, lsc = long silica cell with sinuous edges, pr = prickle, si = silica body. 26) *Calamagrostis ligulata*, from *Cleef 274* (X 940), abaxial epidermis. 27) *Calamagrostis chrysantha*, from *Solomon 4995* (X 300), abaxial epidermis. 28) *Calamagrostis guamanensis*, from *Escalona & S. Gallegos E390* (X 60). 29) *Calamagrostis guamanensis*, from *Escalona & S. Gallegos E390* (X 60). 30) *Calamagrostis chaseae*, from *Briceño 599* (X 240). 31) *Calamagrostis guamanensis*, from *Escalona & S. Gallegos E390*, showing silica content on long silica cells (adaxial surface).

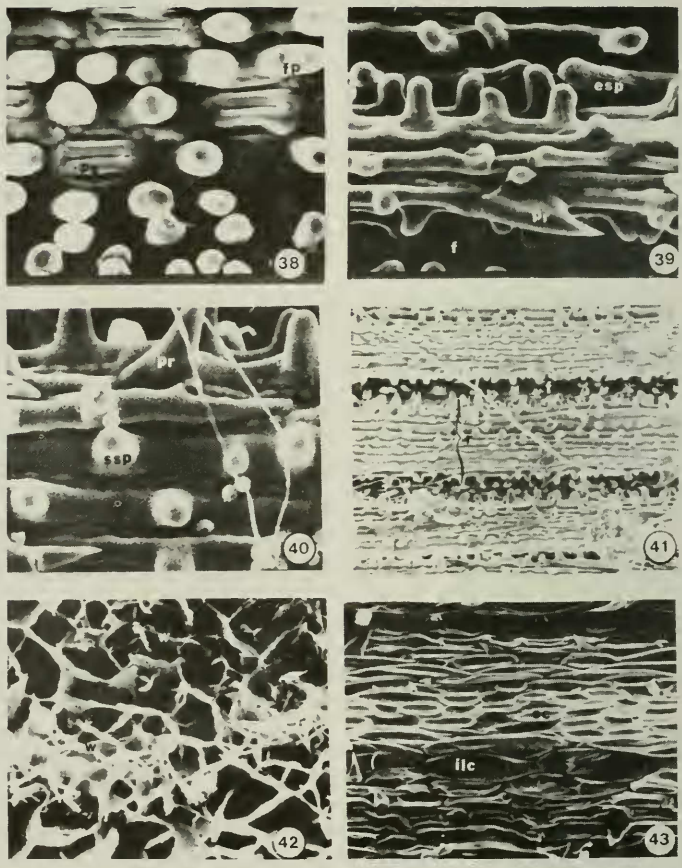








Figures 32-37. Scanning electron micrographs of adaxial epidermis, with X-ray mapping micrographs for the same areas. lsc = silica cell, p = papilla, pr = prickle, w = wax. 32) *Calamagrostis chrysantha*, from *Escalona et al. B549* (X 440). 33) *Calamagrostis chrysantha*, from *Escalona et al. B549* (X 440). 34) *Calamagrostis guamanensis*, from *Laegaard 53861* (X 1800), wax scales covering surface. 35) *Calamagrostis guamanensis*, from *Laegaard 53861* (X 1800). 36) *Calamagrostis chaseae*, from *Luces 292* (X 1000), wax scales covering surface. 37) *Calamagrostis chaseae*, from *Luces 292* (X 1000).



Figures 38-43. Scanning electron micrographs of adaxial epidermis. * = material treated with xylene, # = scanning gamma technique, esp = elongate simple papilla, f = furrow, fp = forked papilla, ilc = inflated long cells with basket type of arrangement, lc = sinuous edged long cell, pr = prickle, ps = parallel sided subsidiary stomata cell, r = rib, sc = silica cell with sinuous edges, ssp = short simple papilla, w = wax. 38) *Calamagrostis chrysantha*, from *Vargas 6577* (X 940) #. 39) *Calamagrostis eminens*, from *Solomon B669* (X 940) *. 40) *Calamagrostis eminens*, from *Lillo 5045* (X 940). 41) *Calamagrostis eminens*, from *Escalona & D. Smith P420* (X 200). 42) *Calamagrostis chaseae*, from *Luces 292* (X 6000). 43) *Calamagrostis guamanensis*, from *Ollgaard & Balslev 10111* (X 1000) *.

subsidiary cells are parallel sided or slightly low dome shaped. They occur at the same level as epidermal cells or below the level of epidermal cells and are associated or unassociated with papillae (Figs. 4, 9, 11, and 17). *Interstomatal cells*: usually one; long, narrow, sometimes with sinuous edges on the adaxial surface and sometimes bearing one papilla per cell (Figs. 8, 12, 14, and 15) or six papillae (Figs. 11, 13, and 38). *Long cells*: may be flat, slightly dome shaped, with or without sinuous edges, narrowly rectangular, or hexagonal, or with sinuous edges on the adaxial surface, sometimes exhibiting papillae (Figs. 10, 12, 13, 14, 16, 18, 22, 23, 39, 40, 41, and 43) or with sinuous edges on the abaxial surface (Figs. 19, 20, and 21), rarely papillated (Fig. 18). Papillae in long cells of the adaxial surface can be globose (Figs. 9, 14, 15, and 17), elongated or forked (1-6 per cell) (Figs. 11, 13, 16, 18, and 32), and exhibited by some species within subsection *Stylagrostis*, such as *Calamagrostis eminens, C. aurea, C. chrysantha*, and *C. ovata*. *Prickles*: robust, tough, sharply pointed or rounded end structures with swollen bases and lignified walls making the leaf surface or margins scabrous. The number, distribution, and form vary from one species to another (Figs. 13, 19, 20, 21, 22, 23, 24, 25, and 39). Sometimes prickles accumulate silica as in *C. chaseae* (Figs. 36 and 37). They are present mostly over the ribs or costal zones adaxially or abaxially (Figs. 22, 23, 24, and 25. Prickles can be found on the intercostal zone and usually have globose bases. *Short cells*: found over the veins; solitary or paired but mostly in short rows. *Silica cells*: mostly costal, longitudinally elongate with round ends and sinuous or nodular outlines on the adaxial epidermis (Figs. 25, 28, 29, 30, and 43), rounded to elongate on the abaxial epidermis (Figs. 26 and 27). *Macrohairs*: short or elongated in the adaxial and/or abaxial epidermis of *C. mollis* (Fig. 22). *Epicuticular wax*: scales over the adaxial surface covering the cuticle (Figs. 14, 16, 17, 34, and 36).

Leaf cross sections were observed using light microscopy, but some structures were also examined under the scanning electron microscope. The pictures were taken orienting the long axis of the leaf horizontally, and scanning both abaxial and adaxial epidermis. It was observed that besides the round and dumbbell shaped silica bodies described for the subfamily Pooideae, the accumulation of silica also occurs in subsection *Stylagrostis* in long cells with sinuous edges (Figs. 34 and 35). Silica also was found in prickles (Figs. 36 and 37) and in papillae (Figs. 32 and 33). The presence of silica in silica cells was detected using energy dispersal X-ray analysis to clarify their structure. The amount of silica in silica cells was detected as shown in caption for Figure 31.

Papillae are not characteristic of the Pooid type of epidermis (Prat 1932, 1936; Metcalfe 1960), but they occur in subsection *Stylagrostis*. They can be present or absent on all long cells, never overarching the stomata (Figs. 11, 13, and 15) in the adaxial epidermis. The development of papillae seems to be a water retention adaptation to environmental conditions in plants inhabiting paramo and puna in the Andes of South America.

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